Amino-acids in the root and bleeding sap of tomato plants

Hofstra, Jurina Johanna

CHAPTER I

INTRODUCTION

In the present paper some experiments concerning amino-acids in the root and bleeding sap of tomato plants are described.

There is not a great deal of literature on amino-acids in the bleeding sap of plants before about 1950, but the bleeding phenomenon itself was the object of much research. Only a brief review of this literature will be given.

The bleeding sap is the liquid that can be collected from the stump of a plant that is decapitated a few centimetres above the root system. In the eighteenth century HALES had already done some bleeding experiments and SACHS (1881) collected in 13 days from one root system of *Helianthus* 1061 ml of bleeding sap, a quantity many times the weight of the root (cited by STOCKING, 1956).

Not all plants show this bleeding. O'Leary showed that the roots of many woody plants, as for example, the conifers, fail to bleed. He found that in some of these plants, however, one isolated root was able to bleed, whereas the whole root system failed to do so. (O'LEARY, 1965).

Many writers have tried to explain the bleeding process and VAN ANDEL (1953) gave a good review of the literature up to about 1952. The bleeding is brought about by two processes; salts are transported into the xylem and water is sucked in osmotically. The most simple formula we can find is that of Sabinin, used by ARISZ and co-workers (1951) and by VAN ANDEL (1953): $b = k (O_x - O_m)$. The rate of exudation (b) depends on the osmotic value of the nutrient solution (O_m), in which the roots are placed and the osmotic value of the xylem sap (O_x), combined with a factor k for the conductivity of the root. The oxygen content, the temperature and the pH of the nutrient solution are other factors which influence bleeding (BROYER, 1951; VAN ANDEL, 1953; O'LEARY, 1965).

We can distinguish between uptake exudation, a process dependent on uptake secretion and thus on uptake of salts, and tissue exudation, dependent on tissue secretion which takes place, even when no salts are taken up from the medium (VAN ANDEL, 1953). Both uptake and tissue secretion are, after Van Andel, inhibited by KCN and other inhibitors. She concludes that both processes partially coincide, their common part possibly being the transport of salts through

the symplast. A stimulation of this transport is found by respiratory substrates (VAN ANDEL, 1953; BROUWER, 1956) and these facts give evidence that this transport of salts is under metabolic control and that somewhere in the sequence of events there must be an active process. (BROUWER, 1965).

BOLLARD (1960), in his review on xylem transport refers to the theories of CRAFTS and BROYER (1938) and of LUNDEGARDH (1950), that the ions are released into the xylem because of a lower oxygen tension in the centre of the root.

The bleeding shows a daily periodicity in rate with a maximum in the day time and a minimum at night. HOFMEISTER, in 1862, described some experiments on this periodicity which he had done with various plants, and he suggested that: "Das periodische Wachsen der Quantität des beim Thränen von Wurzeln ausgeschieden Saftes auf einen Steigen des Turgors des saftreichen Parenchyms des Wurzeln, das Sinken auf eine Erschlaffung des Parenchyms beruhe". SPEIDEL (1939) supposed that a periodical transfer of respiratory substrate or a periodical change in enzyme activity might be the cause of this periodicity. VAÄDIA (1960) related the periodicity not to uptake bleeding but to tissue bleeding - a release of ions previously accumulated. He did not find a periodicity in root respiration or nutrient uptake rates.

At present, there are many things which are not yet clear about the bleeding phenomenon, and today experiments are still being carried out to gain further insight into the mechanisms by which the bleeding is caused (O'LEARY, 1964; TRUBETSKOVA, 1965).

It is, however, possible to use the bleeding sap in order to obtain information about the substances which are transported in the xylem out of the root into the shoot; several experiments have shown that the bleeding sap indeed is xylem sap (STOUT and HOAGLAND, 1939; KRAMER, 1949).

For many years it was supposed that only salts and water are transported in the xylem, but in 1960 BOLLARD stated: "It is no longer possible to picture translocation in plants consisting simply of an upward movement of absorbed mineral elements in the xylem and a downward movement of elaborated nutrients in the phloem. It is now known that organic compounds move in the xylem".

Among these organic compounds are amino-acids and other organic nitrogen compounds.

Even as long ago as the beginning of this century experiments on amino-acids in plant tissue and their relationship to proteins had been carried out. There is an article by PRIANISCHNIKOV, written about 1900, on the synthesis of asparagine and protein turnover in germinating seeds of

Pisum (1899, 1904), and at the same time ZALESKI (1898) worked on the protein synthesis of sprouting bulbs of *Allium cepa*.

By the use of new analytical methods such as paper chromatography and work with isotopes, it proved possible to detect organic nitrogen compounds even in bleeding sap. Thus we find after 1950 plenty of data on the presence of amino-acids in the bleeding sap, and at about this time theories about the role of the root in the plant were changing.

Formerly it was supposed that the root served only for the uptake of salts and water out of the soil. Now, since Kursanov's work, it appears that the root is metabolically one of the most active organs of the plant, which absorbs elements from the soil, assimilates them and delivers their functional products to the shoot. A considerable portion of these products is utilized by the growing regions of the root for the synthesis of protein and nucleic acids, and some rise in the transpiration stream to the shoot, to the growing regions of the shoot and to the developing fruits (KURSANOV, 1957).

One of the first research-workers on amino-acids in the xylem sap is Bollard. He blew out the xylem sap, which was present in a part of the stem, and used this sap in his experiments. He supposed, like Kursanov, that the amino-acids are synthesized in the root, out of which some are transported to the shoot, where they are assimilated into protein, but it may also be possible that some of the amino-acids in the bleeding sap originate from a breakdown of protein (BOLLARD, 1957).

He examined many species of plants; glutamine, asparagine and their acids proved to be the most important organic nitrogen compounds of the xylem sap. In other plants, however, he found instead allantoine or citrulline, the function of which was not clear to him (BOLLARD, 1957, 1960). Mothes and co-workers suggested that all these organic nitrogen compounds, as glutamine, asparagine, citrulline and allantoine, function as a means of transporting and storing nitrogen. Their synthesis seems to follow a certain carbohydrate economy, as each molecule, especially allantoine and citrulline, contains many nitrogen atoms. They can be stored in stem and roots as, for example, citrulline is stored during the winter in the Betulaceae, and are easily transported and metabolized in the shoot (MOTHES, 1961).

Alkaloids, which are synthesized in the root, as nicotine in tobacco, may have the same function (MOTHES, 1955, DAWSON, 1942).

Some interesting taxonomic relations are described by

MOTHES (1961). In the bleeding sap of the Betulaceae citrulline is found as the most important organic nitrogen compound, but in the Fagaceae we find glutamine and asparagine. With the Papaveraceae we find the Fumaroideae which contain acetylornithine and the Papaveroideae, which contain glutamine.

REINBOTHE (1961) worked on the biosynthesis of allantoine, found in the root and bleeding sap of the Boraginaceae; and MOTHES and REINBOTHE gave a review of the presence of ureides and guanidines in plants (1962).

Van Die published various data on the composition of the bleeding sap of the tomato plant. He concluded that the site of synthesis of amino-acids was in the youngest roots, where they are synthesized from ammonium and keto-acids. Glutamine is the major organic nitrogen compound of the bleeding sap (VAN DIE, 1958, 1960, 1962).

The nitrogen fixation in the nodules of the Leguminosae, as *Pisum* and *Lupinum*, was examined by Pate and collaborators in bleeding and other experiments (PATE, 1962; PATE and WALLACE, 1964, PATE and GREIG, 1964). The carbohydrates, transported to the root, were labelled by means of $C^{14}O_2$, given to the leaves. The bleeding sap of these roots was fed to the isolated shoot of other plants in order to obtain some information about the distribution and assimilation of the products which are transported out of the root (PATE *e.a.*, 1965).

Other experiments were carried out with N^{15} on nodules of the non-leguminous plants *Myrica* and *Alnus* (BOND, 1956; LEAF *e.a.*, 1959).

Bleeding sap was used for the control of the effectivity of *Rhizobia* strains, inoculated on *Pisum* (WIERINGA and BAKHUYS, 1957).

POTAPOV and CSEH (1952) examined the bleeding sap of *Cucurbita* and *Zea Mays* for agricultural purposes, for information about the condition of the soil and the root system.

In experiments on the influence of the nitrogen source on the amino-acid composition of the plant, examination of the bleeding sap can be very useful (e.g. POSKUTA, 1961; WEISSMAN, 1964).

In the present experiments the relation between the amino-acids in the bleeding sap and in the root and the influence of the bleeding process on the amino-acid content of the root were studied.

SUMMARY

1. In experiments with tomato plants the relationship between the amino-acids of root and bleeding sap were studied.
2. The amino-acid content of the root is variable and shows a daily periodicity with a minimum in the daytime.
3. A positive correlation exists between the amino-acid content of the root and the amino-acid concentration of the bleeding sap, but the amino-acid and the sugar content of the root are negatively correlated.
4. Succinic acid given to the root, may act not only as an amino-acid precursor but also as an energy source and as such it may, if sugar is limiting, increase the bleeding rate.
5. The nitrogen source of the nutrient solution is important; ammonium increases the amino-acid content of the root more than nitrate does.
6. Both the transpiration stream and the phloem stream influence the amino-acid content of the root; the transpiration stream by transporting amino-acids out of the root and the phloem stream by supplying sugars to the root.
7. Fruiting decreases vegetative growth; the amino-acid and sugar content of the root is decreased in fruiting plants and these facts influence in their turn the amino-acids, transported in the bleeding sap.
8. Environmental conditions, such as the amount of light, given to the shoot, the composition and the amount of nutrient solution, influence the sugar and keto-acid content of the root and, consequently, the amino-acid content.